

CLAIMS

- Sub B1
1. A yeast cell containing the *SRB1/PSA1* gene and the *PKC1* gene or functional derivatives thereof each operatively linked to a heterologous inducible promoter.
 2. The yeast cell according to claim 1 wherein the yeast cell is a strain of *Saccharomyces cerevisiae*.
 3. The yeast cell according to claim 1 wherein the yeast cell is a strain of *Pichia pastoris*, *Hansenula polymorpha* or *Kluyveromyces lactis*.
 4. The yeast cell according to ~~any one of claims 1-3~~ wherein at least one of the genes or functional derivatives thereof is operatively linked to a methionine regulated promoter.
 5. The yeast cell according to claim 4 wherein the methionine regulated promoter is *pMET3*.
 6. The yeast cell according to claim 5 wherein the *PKC1* gene or functional derivative thereof operatively linked to an inducible promoter is derived from a recombinant vector selected from *pRS316-pMET3-PKC1*, *pRS316-F₁F₂-pMET3-PKC1* or *pRS316-F₁F₂-TRP1-pMET3-PKC1*.
 7. The yeast cell according to claim 5 wherein the *SRB1/PSA1* gene or functional derivatives thereof operatively linked to an inducible promoter is derived from the recombinant vector *SRB1.9e*.
 8. The yeast cell according to claim 7 wherein the *PKC1* gene or functional derivatives thereof operatively linked to an inducible promoter is derived from a
- a
- 09700492.010501
- Sub B2

recombinant vector selected from pRS316-pMET3-~~PKC1~~, pRS316-F₁F₂-pMET3-~~PKC1~~ or pRS316-F₁F₂-TRP1-pMET3-~~PKC1~~.

9. A method of regulating yeast cell lysis comprising:

- (i) growing yeast cells containing the *SRB1/PSA1* gene and the *PKC1* gene or functional derivatives thereof each operatively linked to an inducible promoter in a growth medium which activates the inducible promoter such that *SRB1/PSA1* and *PKC1* are expressed from said cells; and
- (ii) when lysis is required, growing the cells in a modified growth medium which represses *SRB1/PSA1* and *PKC1* expression such that cell lysis is induced.

10. The method according to claim 9 wherein the yeast cells are cells according to any one of claims 1 - 8.

11. The method according to claim 9 ~~or 10~~ wherein the inducible promoter is *pMET*, the growth medium is methionine-free and the modified growth medium contains methionine.

12. The method according to claim 11 wherein the modified medium contains from between 0.05mM and 20mM methionine.

13. A method of isolating protein from yeast cells comprising growing cells and inducing lysis according to ~~any one of claims 9 - 12~~ and separating the protein released from the lysed yeast cells from yeast cell debris / ghosts.

14. The method according to claim 13 for isolating recombinant proteins expressed from genetically engineered yeast cells.

B2
conc.

09700493.01000000

Sub 103

a

15. A method of regulating yeast cell flocculation comprising:
- (i) growing yeast cells containing the *PKC1* gene or a functional derivative thereof operatively linked to an inducible promoter in a growth medium which activates the inducible promoter such that *PKC1* is expressed; and
 - (ii) when flocculation is required, growing the cells in a modified growth medium which represses *PKC1* expression such that flocculation is induced.
16. The method according to claim 15 wherein the yeast cells are a strain of *Saccharomyces cerevisiae*
17. The method according to claim 15 wherein the yeast cells are a strain of *Pichia pastoris*, *Hansenula polymorpha* or *Kluyveromyces lactis*.
18. The method according to ~~any one of claims 15 to 17~~ wherein the *PKC1* gene or functional derivative thereof is operatively linked to a methionine regulated promoter.
19. The method according to claim 18 wherein the methionine regulated promoter is *pMET3*.
20. The method according to claim 19 wherein the yeast cells contain the *PKC1* gene or functional derivative thereof operatively linked to *pMET3* derived from a recombinant vector selected from *pRS316-pMET3-PKC1*, *pRS316-F₁F₂-pMET3-PKC1* or *pRS316-F₁F₂-TRP1-pMET3-PKC1*.
21. The method according to claim 20 wherein the yeast cells are ZO-126.

a 22. The method according to ~~any one of claims 15 to 20~~ wherein the yeast cells are ZO123 or ZO124 transformed with the *PKC1* gene or functional derivative thereof operatively linked to an inducible promoter.

a 23. The method according to ~~any one of claims 15 to 22~~ for increasing the sedimentation of yeast cells or cell ghosts / debris from a medium within which the yeast cells are grown.

24. A method of fermentation comprising growing yeast cells containing the *SRB1/PSA1* gene or functional derivative thereof operatively linked to a heterologous promoter in a growth medium in which *SRB1/PSA1* expression is regulated by the heterologous promoter whereby said cells flocculate.

25. The method according to claim 24 wherein the yeast cell is a strain of *Saccharomyces cerevisiae*

26. The method according to claim 24 wherein the yeast cell is a strain of *Pichia pastoris*, *Hansenula polymorpha* or *Kluyveromyces lactis*.

27. The method according to ~~any one of claims 24 - 26~~ wherein the *SRB1/PSA1* gene or functional derivative thereof is operatively linked to a methionine regulated promoter.

28. The method according to claim 27 wherein the methionine regulated promoter is pMET3.

29. The method according to claim 28 wherein the *SRB1/PSA1* gene or functional derivatives thereof operatively linked to an inducible promoter is derived from the recombinant vector SRB1.9e.

30. The method according to claim 29 wherein the yeast cells are ZO-125.

31. The method according to claim 29 wherein the yeast cells are FY23SRB1/MET3.

32. A method of fermentation comprising growing yeast cells containing the *SRB1/PSA1* and *PKC1* gene or functional derivatives thereof operatively linked to a heterologous promoter in a growth medium in which *SRB1/PSA1* and *PKC1* expression is regulated by the heterologous promoter whereby said cells flocculate.

33. The method according to claim 32 wherein the cells are cells according to any one of claims 1 - 8.

34. The method according to claim 32 wherein the cells contain the *PKC1* gene or a functional derivative thereof operatively linked to a heterologous inducible promoter and the *SRB1/PSA1* gene or a functional derivative thereof operatively linked to a heterologous promoter.

35. A yeast cell containing the *PKC1* gene or functional derivatives thereof operatively linked to a heterologous inducible promoter selected from the group consisting of:

(i) ZO124 transformed with pRS316-pMET3-*PKC1*, pRS316-F₁F₂-pMET3-*PKC1* or pRS316-F₁F₂-TRP1-pMET3-*PKC1*;

(ii) ZO123 transformed with pRS316-pMET3-*PKC1* or pMET3-*PKC1* containing fragments derived from pRS316-F₁F₂-pMET3-*PKC1* or pRS316-F₁F₂-TRP1-pMET3-*PKC1*; and

(iii) yeast strain ZO-126.

36. A yeast cell containing the *SRB1/PSA1* gene or functional derivatives thereof operatively linked to a heterologous promoter.

11-08-2000

44

Sub B5

37. A yeast cell containing the *PKC1* gene or a functional derivative thereof operatively linked to a heterologous inducible promoter and the *SRB1/PSA1* gene or a functional derivative thereof operatively linked to a heterologous promoter.

a

38. A yeast cell according to ~~any one of claims 35~~³⁶ / 37 wherein the promoter or promoters is/are *pMET3*.

09700492.010504
T05070.26400260